Saxeibacter lacteus gen. nov., sp. nov., an actinobacterium isolated from rock

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A Gram-positive, cream-coloured, non-motile, rod-shaped actinomycete, designated DLS-10^T, was isolated from a rock collected on the peak of Darangshi Oreum (a small mountain 300 m above sea level) in Jeju, Republic of Korea. The temperature and pH ranges for growth were 4–37 °C and pH 5.1–9.1. The organism grew at NaCl concentrations up to 3% and had the following chemotaxonomic characteristics: meso-diaminopimelic acid was the diagnostic diamino acid in the cell-wall peptidoglycan, the polar lipids included diphosphatidylglycerol, phosphatidylmethylethanolamine and two unknown phospholipids, the major menaquinone was MK-8(H₄) and the major fatty acids were anteiso-C_{15:0}, C_{16:0}, iso-C_{16:0} and anteiso-C_{17:0}. The DNA G + C content was 74.3 mol%. Phylogenetic analyses based on 16S rRNA gene sequences revealed that the organism was related to members of the family *Nakamurellaceae* in the suborder *Frankineae*. The most closely related described organisms were the type strains of *Humicoccus flavidus* (97.1% sequence similarity) and *Nakamurella multipartita* (95.2%). On the basis of the morphological, cultural, physiological, chemotaxonomic and phylogenetic evidence presented here, the organism represents a novel genus and species of the family *Nakamurellaceae*, for which the name *Saxeibacter lacteus* gen. nov., sp. nov. is proposed. The type strain of *Saxeibacter lacteus* is DLS-10^T (=KCTC 19285^T =DSM 19367^T).

The family *Microsphaeraceae* was originally proposed by Stackebrandt *et al.* (1997) to encompass the only (and type) genus, *Microsphaera* (Yoshimi *et al.*, 1996), on the basis of 16S rRNA gene sequences. The nomenclature of the family and genus were later changed to *Nakamurellaceae* and *Nakamurella*, respectively (Tao *et al.*, 2004). The family *Nakamurellaceae* belongs phylogenetically to the suborder *Frankineae* and currently consists of two genera, *Nakamurella* (Tao *et al.*, 2004) and *Humicoccus* (Yoon *et al.*, 2007).

Strain DLS-10^T was isolated from a rock sample collected from the peak of Darangshi Oreum (a small mountain 300 m above sea level) in Jeju, Republic of Korea. Rock samples (1 g) were ground to powder using a pestle and suspended in 10 ml sterilized, distilled water. Aliquots of serial dilutions of the sample were transferred to starch-casein agar (1% soluble starch, 0.03% casein, 0.2% KNO₃, 0.2% NaCl, 0.2% KH₂PO₄, 0.002% CaCO₃, 0.005% MgSO₄·7H₂O, 0.001% FeSO₄·7H₂O and 1.8% agar; pH 7.2) and incubated at 30 °C for 7 days. The isolate

was maintained as a 20% glycerol suspension at –20 and –80 °C. For physiological and chemotaxonomic comparisons, *Humicoccus flavidus* KCTC 19127^T was grown on trypticase soy agar (TSA; Difco) at 30 °C.

Cell morphology and motility were observed by using phase-contrast microscopy. The presence of flagella was checked with a transmission electron microscope (JEM-1010; JEOL), using cells negatively stained with 2% phosphotungstic acid. Colony morphology and pigmentation were observed on a TSA plate incubated for 5 days at 30 °C. Growth was tested at 0, 4, 10, 30, 37 and 42 °C and at initial pHs in the range 4.1–12.1 (with increments of 1 pH unit, adjusted using HCl or NaOH prior to sterilization). Tolerance of NaCl was determined on TSA amended with 1–9% NaCl (w/v). The following physiological tests were performed as described previously (Seo & Lee, 2006): Gram staining, utilization of carbohydrates, oxidase and catalase activities and hydrolysis of aesculin, casein, elastin and starch, hypoxanthine, DL-tyrosine and xanthine. Hydrolysis of chitin and cellulose was checked on TSA supplemented with 0.5% (w/v) colloidal chitin and 0.5% (w/v) CM-cellulose (Sigma), respectively. Anaerobic growth was tested using the GasPak EZ Anaerobe Pouch System (BD) according to the manufacturer's instructions. Acid production from carbohydrates was determined using OF basal medium (Difco) that included each filter-sterilized compound at a final concentration of 1%

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DLS-10^T is AM778124.

A transmission electron micrograph of cells of strain DLS-10^T and details of the cellular fatty acid profiles of strain DLS-10^T and its phylogenetic neighbours are available as supplementary material with the online version of this paper.

(w/v). Other physiological and biochemical properties were tested using API 20E and API ZYM strips (bioMérieux) according to the manufacturer's instructions. For these tests, cells were grown on TSA for 3 days at 30 °C and suspended in a solution of 0.85 % (w/v) NaCl. The cells of strain DLS-10^T were Gram-positive, aerobic, catalase-positive, oxidase-negative, non-mycelium-forming, non-motile, short rods (see Supplementary Fig. S1, available in IJSEM Online). The data on cultural, physiological and biochemical properties are summarized in the species description and Table 1.

DNA isolation and PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Seo & Lee, 2006). The CLUSTAL_X program (Thompson *et al.*, 1997) was used for multiple alignment of the sequence determined in this study with the corresponding sequences retrieved from public databases. Phylogenetic analyses were performed using the DNAML, DNAPARS and NEIGHBOR programs contained in the PHYLIP package (Felsenstein, 1993). A phylogenetic tree was constructed

using the model of Jukes & Cantor (1969) and the neighbour-joining method (Saitou & Nei, 1987). A bootstrap analysis (Felsenstein, 1985) was performed with 1000 replicated datasets.

An almost-complete 16S rRNA gene sequence (1399 nt) of strain DLS-10^T was compared with those of representatives of the suborder *Frankineae* and related taxa. The results of phylogenetic analyses based on 16S rRNA gene sequences showed that the organism formed a consistent clade with members of the family *Nakamurellaceae* (Fig. 1), with high bootstrap support (100 %). The most closely related described neighbours were *H. flavidus* DS-52^T (97.1 % 16S rRNA gene sequence identity) and *Nakamurella multipartita* JCM 9543^T (95.2 %). Strain DLS-10^T shared a 16S rRNA gene sequence similarity of 99.1 % with respect to an undescribed strain, Actinomycetales bacterium Gsoil 972.

Cell biomass of strain DLS-10^T and *H. flavidus* KCTC 19127^T for chemotaxonomic analyses was obtained from cultures grown in trypticase soy broth (Difco) at 30 °C. Analysis of the cell-wall peptidoglycan (Staneck & Roberts,

Table 1. Differential characteristics between strain DLS-10^T and related taxa of the family *Nakamurellaceae*

Data for *H. flavidus* KCTC 19127^T were taken from Yoon *et al.* (2007) and this study, and data for *N. multipartita* JCM 9543^T were taken from Yoshimi *et al.* (1996) and Yoon *et al.* (2007). All showed growth at 10–32 °C, pH 5.1–8.1 and in the presence of up to 3 % (w/v) NaCl. All were positive for the Gram stain, catalase activity, hydrolysis of aesculin and utilization of D-galactose and glycerol. All were negative for motility, oxidase activity, hydrolysis of DL-tyrosine and xanthine, anaerobic growth and utilization of acetate. +, Positive; –, negative; w, weak; ND, not described.

Characteristic	Strain DLS-10 ^T	<i>H. flavidus</i> KCTC 19127 ^T	<i>N. multipartita</i> JCM 9543 ^T
Cell morphology	Rod	Coccus	Coccus
Colony colour	Cream	Light yellow	White
Growth at/in:			
4 °C	w	+	–
37 °C	+	–	–
pH 9.1	+	–	+
4 % (w/v) NaCl	–	+	+
Nitrate reduction	+	–	ND
Hydrolysis of:			
Casein	–	–	+
Hypoxanthine	–	–	+
Starch	–	+	+
Utilization of:			
D-Fructose, D-glucose, D-lactose, maltose, D-mannose, sucrose, D-xylose, D-mannitol, D-sorbitol	–	+	+
Malate, succinate	–	+	–
Acid production from sucrose, D-xylose, glycerol	–	+	ND
Polar lipids*	DPG, PME	DPG, PE, PME, PI	DPG, PE, PME
Whole-cell sugars†	Glc, Rha, Xyl, Rib, Ara	Gal, Rha, Xyl, Man	Glc, Rha, Xyl, Man
Major fatty acids	anteiso-C _{15:0} , C _{16:0} , iso-C _{16:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , C _{16:0} , C _{17:0} , iso-C _{15:0}	iso-C _{16:0} , C _{18:1} ω9c
DNA G + C content (mol%)	74.3	72.6	67.5

*DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

†Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose. Sugars are listed in decreasing order of abundance.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain DLS-10^T within the radiation of the family *Nakamurellaceae* and related taxa. Asterisks indicate branches that were also supported by both the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analysis was based on a neighbour-joining analysis of 1000 resampled datasets (values above 50% are shown at nodes). Bar, 0.01 substitutions per nucleotide position.

1974) of strain DLS-10^T showed that it contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The whole-cell sugars of the isolate were glucose, rhamnose, xylose, ribose and arabinose (in decreasing order of abundance), as analysed by gas chromatography (Saddler *et al.*, 1991). The menaquinone profile of strain DLS-10^T, as determined using HPLC (Kroppenstedt, 1985), contained MK-8(H₄), MK-9(H₀), MK-7(H₀) and MK-8(H₂) (peak ratio, 66:12:12:10). It was reported by Yoon *et al.* (2007) that the predominant menaquinones in *H. flavidus* KCTC 19127^T are MK-8(H₄) and MK-9(H₄), but the latter was not detected in our study. Analysis of the polar lipids by TLC (Minnikin *et al.*, 1977) revealed that diphosphatidylglycerol, phosphatidylmethylethanolamine and two unknown phospholipids were present in extracts of the isolate, but phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol were not present. In contrast, *H. flavidus* KCTC 19127^T was shown to contain a considerable amount of phosphatidylinositol in addition to the polar lipids reported previously (Yoon *et al.*, 2007). The G+C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989): the value for strain DLS-10^T was 74.3 mol%.

The cellular fatty acid compositions of strain DLS-10^T and *H. flavidus* KCTC 19127^T were determined using the Sherlock Microbial Identification System (version 6.0; MIDI) according to the manufacturer's instructions, using

cells grown on TSA at 30 °C for 5 days. The predominant fatty acids of the isolate were anteiso-C_{15:0} (25.8%), C_{16:0} (19.6%), iso-C_{16:0} (15.7%) and anteiso-C_{17:0} (15.0%), while those of *H. flavidus* KCTC 19127^T were anteiso-C_{15:0} (27.7%), C_{16:0} (13.7%), C_{17:0} (13.0%) and iso-C_{15:0} (10.7%). Strain DLS-10^T differed significantly from *H. flavidus* KCTC 19127^T in the relative amounts of C_{17:0}, iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0} and with respect to the absence/presence of C_{17:1}ω6c and anteiso-C_{15:0} 2-OH (see the cellular fatty acid profiles in Supplementary Table S1). *N. multipartita* DSM 44233^T is clearly distinguishable from strain DLS-10^T in that the predominant fatty acids in the former are iso-C_{16:0} and C_{18:1}ω9c (Yoon *et al.*, 2007).

In addition to the major differences in the fatty acid profile, strain DLS-10^T can be readily differentiated from *H. flavidus* and *N. multipartita* on the basis of cell morphology and colony pigmentation. Cells of the isolate are short rods and produce cream-coloured colonies, whereas the type strains of *H. flavidus* and *N. multipartita* are coccoid in shape and produce light yellow- or white-coloured colonies. Strain DLS-10^T also differs from the type strains of *H. flavidus* and *N. multipartita* in that it lacks phosphatidylethanolamine and phosphatidylinositol in the polar lipid profile. Other differential characteristics for strain DLS-10^T and the type strains of *H. flavidus* and *N. multipartita* are summarized in Table 1.

On the basis of morphological, cultural, physiological and chemotaxonomic data, as well as phylogenetic distinctness, strain DLS-10^T represents a novel genus and species of the family *Nakamurellaceae*, for which the name *Saxeibacter lacteus* gen. nov., sp. nov. is proposed.

Description of *Saxeibacter* gen. nov.

Saxeibacter (Sa.xe.i.bac'ter. L. adj. *saxeus* of rock; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Saxeibacter* a rod isolated from rock).

Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive, non-mycelium-forming, non-motile, short rods (0.4–0.7 × 0.9–1.0 μm). The isomer of diamino acid in the cell-wall peptidoglycan is *meso*-diaminopimelic acid. The whole-cell sugars are glucose, rhamnose, xylose, ribose and arabinose. The major menaquinone is MK-8(H₄). The polar lipids include diphosphatidylglycerol, phosphatidylmethylethanolamine and two unknown phospholipids. The predominant fatty acids are anteiso-C_{15:0}, C_{16:0}, iso-C_{16:0} and anteiso-C_{17:0}. The DNA G+C content of the type strain of the type species is 74.3 mol%. On the basis of 16S rRNA gene sequence studies, the genus belongs to the family *Nakamurellaceae* in the suborder *Frankineae*. The type species for the genus is *Saxeibacter lacteus*.

Description of *Saxeibacter lacteus* sp. nov.

Saxeibacter lacteus (lac.te'us. L. masc. adj. *lacteus* milk-coloured, milky).

Colonies are cream-coloured, circular, smooth and convex, reaching 0.5–1.0 mm in diameter. The morphological and chemotaxonomic characteristics are the same as those given in the genus description. The temperature and pH ranges for growth are 4–37 °C and pH 5.1–9.1. No growth occurs at 0 or 42 °C, at pH 4.1 or above pH 10.1. Grows at NaCl concentrations up to 3%; does not grow in the presence of 4% NaCl. Positive for hydrolysis of DNA; negative for hydrolysis of chitin, CM-cellulose and elastin. Nitrate is reduced to nitrite. The following tests in the API 20E system are negative: H₂S production, utilization of citrate, indole production and Voges–Proskauer reaction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase. In the API ZYM system, tests for leucine arylamidase, valine arylamidase, β -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase are positive. Weakly positive for esterase lipase (C8) and acid phosphatase. Negative for alkaline phosphatase, esterase (C4), lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, β -glucosidase and α -fucosidase. L-Arabinose, myo-inositol and methyl α -D-mannoside are utilized as sole carbon and energy sources, but benzoate, cellobiose, citrate, dextran, D-dulcitol, meso-erythritol, formate, methyl α -D-glucoside, inulin, melezitose, raffinose, L-rhamnose, salicin, L-sorbose, DL-tartrate, trehalose and D-xylitol are not used. Acid is produced from D-galactose. Other physiological properties are given in Table 1. The predominant fatty acids are anteiso-C_{15:0} (25.8%), C_{16:0} (19.6%), iso-C_{16:0} (15.7%) and anteiso-C_{17:0} (15.0%). The DNA G + C content is 74.3 mol%.

The type strain, DLS-10^T (=KCTC 19285^T =DSM 19367^T), was isolated from a rock sample collected from the peak of Darangshi Oreum (a small mountain, 300 m above sea level) in Jeju, Republic of Korea.

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